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Characteristics of Na^+/K^+-ATPase mediated proton current in Na^+- and K^+-free extracellular solutions. Indications for kinetic similarities between H^+/K^+-ATPase and Na^+/K^+-ATPase

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Abstract

The Na^+/K^+-ATPase of an ouabain-resistant mutant of *Torpedo californica* and of rat was expressed in *Xenopus* oocytes by microinjection of mRNA coding for the α- and the β-subunit of the protein. Electrophysiological measurements were performed by means of the extracellular giant-patch clamp technique. The pump currents were analyzed in nominal absence of extracellular Na^+ and K^+ ions. Under these conditions strongly inward rectifying, ouabain-sensitive current was detected with reversal potentials depending on extracellular pH. Presence or absence of intracellular Na^+ or K^+ ions had nearly no effect on the inward currents, removal of intracellular ATP caused their inhibition. The reversal potentials of the currents generated by the rat pump was shifted by 82.7 ± 5.4 mV per 10-fold increase of extracellular proton concentration. This refers to an effective valency of 0.71 ± 0.05. In the absence of a transmembrane proton gradient the pump current reversed at -64.2 ± 4.4 mV. These findings are not compatible with a proton conducting channel formed by the pump molecule (Wang and Horisberger (1995) Am. J. Physiol. CP 37, C590–595). Therefore, a kinetic model based on the Post-Albers scheme with a modification derived from the reaction scheme of the gastric H^+/K^+-ATPase is proposed. Together with voltage-dependent binding of extracellular protons, this model is compatible with the observed pump currents.

Keywords: ATPase, Na^+/K^+-; ATPase, H^+/K-; Patch-clamp; Current–voltage relationship; Proton transport; (*Xenopus* oocyte)

1. Introduction

The Na^+/K^+-ATPase or sodium pump belongs to the group of electrogenic P-type ATPases and can be found in the membrane of most animal cells. Under physiological conditions the sodium pump carries per transport cycle 3 Na^+ ions out of and 2 K^+ ions into the cell under consumption of 1 ATP molecule. Under unphysiological conditions several other transport modes have been described and also binding and transport of extracellular protons replacing K^+ ions was discussed [1,2].

Theoretically, the pump process should cease if extracellular Na^+ and K^+ ions are removed. Nevertheless, a small ouabain-sensitive, inward rectifying current has been detected [3,4]. In a recently published paper this inward current has been analysed in more detail by means of the two-electrode voltage clamp method [5]. The authors attributed their findings to a proton-permeable, passive pathway that is formed by the sodium pump protein.

Because of the poor control of the intracellular ion composition and especially of intracellular pH by using the two-electrode method, a giant patch-clamp technique in inside- and outside-out configurations was used to analyze this current [6,7]. With this method it is possible to have perfect control over the medium at both sides of the membrane. This methodological advantage gave the opportunity to concentrate on the dependence of pump currents on intra- and extracellular substrate concentrations (i.e., ATP, Na^+, K^+ and H^+) and membrane potential. The pump species used in the presented study are the sodium pump of *Torpedo* electroplax (ouabain-resistant mutant of the α-subunit together with the

Abbreviations: ORI, oocyte Ringer solution; MS222, *m*-aminobenzoic acid ethyl esther methane sulfonate; Mops, morpholinopropanesulfonic acid; EGTA, ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; TEA, tetraethylammonium; Tris, Tris(hydroxymethyl)aminomethane.

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β-subunit) and of rat (α_1- and mouse β_1-subunit). The rat pump was used to study the pH-dependence of the reversal potentials because this pump species exhibits larger inward current than the *Torpedo* pump and gave, therefore, better resolution for determination of the reversal potentials. Qualitatively both pump species have the same characteristics with respect to the pump currents in Na^+- and K^+-free extracellular solutions. For simplification, the pump currents that can be detected in absence of extracellular Na^+ and K^+ ions are termed 'proton pump currents', although other ion species could be co- or counter-transported together with protons.

2. Materials and methods

2.1. Oocytes and injection of MRNA

The way of maintenance of toads, oocyte treatment and microinjection of mRNA into the oocytes resembles the procedures that have been described earlier [8–10] and is, therefore, described only briefly.

Females of the clawed toad *Xenopus laevis* were anaesthetized in aqueous solution (2 mg/ml) of MS222 (Sandoz, Basel, Switzerland). Parts of the ovary were removed through a small seizure and treated with collagenase (Sigma) to remove the surrounding tissue. Directly before the giant-patch experiment the vitelline layer was removed manually after short incubation in hyperosmolaric medium (ORI, see below) +300 mOsm Sucrose). For the expression of α- and β-subunits in the oocyte membrane 25 ng mRNA of each subunit were injected into the oocyte cytoplasm. Measurements were started after 2 to 3 days of incubation in ORI at 18°C.

2.2. Electrophysiological measurements

Sodium pump currents were studied using the giant-patch clamp technique. With our modification of the technique it is possible to obtain stable gigaseals in inside- and also easily in outside-out configuration with electrode openings up to 50 μm [7]. Small tipped patch pipettes were prepared from borosilicate glass capillaries (OD 1.5 mm, ID 0.87 mm (Hilgenberg, Malsfeld, Germany)) on a standard electrode puller (Narishige PP83). To get large opening of the pipette tips, the electrodes were shortened to the desired diameter by microforge treatment (Narishige MF79). Except for heatpolishing of the pipette tips, no additional modifications (e.g., coating) were done.

To extract pump currents from total membrane currents, current–voltage dependencies (*I–V*-dependencies) were determined in the presence of 10 μM and 10 mM extracellular ouabain. The endogenous pump was inhibited by adding 10 μM ouabain to all extracellular solutions without affecting the expressed ouabain-resistant pumps. The expressed pump currents were then defined as the differences between *I–V*-curves with 10 μM or 10 mM extracellular ouabain under otherwise identical conditions. The *I–V*-dependencies were determined by changing the membrane potential stepwise from −150 mV to +60 mV for a duration of 100 ms. The currents were filtered at 1 kHz and sampled at 2 kHz (EPC-9, HEKA Elektronik, Lambrecht, Germany). The sampled values of the last 20 ms of each record were averaged and the normalized currents were plotted against the corresponding membrane potential. For the outside-out experiments the proton currents were normalized to the K^+-activated outward pump current (5 mM K^+) at −50 mV, for the inside-out experiments the proton currents were normalized to the inward pump current at pH 6.5 and −150 mV. The respective mean currents of a set of data are included in the figure legends. All experiments were performed at 22°C, the temperature of the solutions was kept constant via a Peltier device.

2.3. Solutions

The composition of the ORI medium was (in mM): 110 NaCl, 2 KCl, 2 $CaCl_2$, 5 Mops/NaOH (pH 7.4). The basic 'intracellular' solution was: 10 $MgCl_2$, 5 Na_2ATP, 5 EGTA, 25 NaOH, 5 Mops (pH 7.5). This solution was modified with respect to ATP/ADP and Na^+/K^+ content depending on the experimental intention. The basic 'extracellular' solution was: 30 TEA-Cl, 5 $BaCl_2$, 10 μM or 10 mM ouabain, 5 Mops/Tris (pH 6.5, 7.5 or 8.5). Solution exchange during the experiment was performed via a thin tube ($d = 150$ μm) in the vicinity of the membrane patch.

3. Results

3.1. Reversal potentials of pump currents in the absence of extracellular Na^+ and K^+ ions. Dependence on extracellular pH

To study the mechanism leading to pump currents in the absence of extracellular Na^+ and K^+ ions, the voltage dependencies of pump currents at different extracellular pH and constant intracellular pH of 7.5 (outside-out configuration,

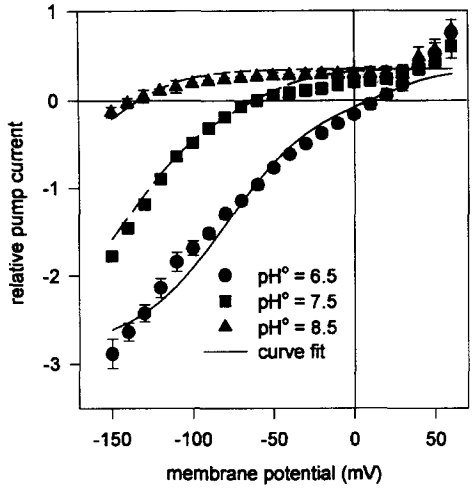

Fig. 1. Voltage dependence of pump currents (ouabain-sensitive currents) in Na^+- and K^+-free extracellular solution at different extracellular pH (mean ± S.E.M., $n = 6$, outside-out patch, mean diameter 35 μm, rat pump). The currents were normalized to the K^+ activated outward pump current at −50 mV measured before each experiment in the same membrane patch (mean current 22.0 ± 3.1 pA). The overlaid curves show the result of a nonlinear least square fit of the model equations to the experimental data (see Appendix and Section 4). A simultaneous fit of Eq. (2) to the voltage dependence of pump currents at different extracellular pH was performed assuming voltage-dependent extracellular binding of protons in analogy to the voltage dependence of extracellular K^+ activation [11]. For the fitted parameters see Table 1.

mean tip diameter 35 μm) were analyzed. *I–V*-dependencies with 10 μM or 10 mM extracellular ouabain were recorded at each of three different extracellular pH values. The voltage dependence of pump currents at external pH 6.5, 7.5 and 8.5 are shown in Fig. 1. In Fig. 2 the reversal potentials (taken from Fig. 1) are plotted versus the transmembrane pH gradients on a logarithmic scale. The following modified Nernst equation was arbitrarily fitted to the data (straight line in Fig. 2):

$$U_{Rev} = U_o + \frac{RT}{z^* F} \cdot \ln\left(\frac{[H^+]^o}{[H^+]^i}\right) \quad (1)$$

At pH 7.5 on both sides of the membrane the fit of Eq. (1) to the data yields a reversal potential $U_o = -58 \pm 5.4$ mV for the ouabain-sensitive current. The slope of the regression line in Fig. 2 is 82.7 ± 5.4 mV per 10-fold increase of the proton gradient. The pH dependence of the currents indicates that indeed protons are transported by the sodium pump, in line with the conclusions of Wang and Horisberger [5]

3.2. Activation by intracellular ATP

All described transport modes of the Na^+/K^+-ATPase except the reversed mode are known to work only in the presence of intracellular ATP [1]. To determine whether the proton pump current also depends on intracellular ATP, experiments in

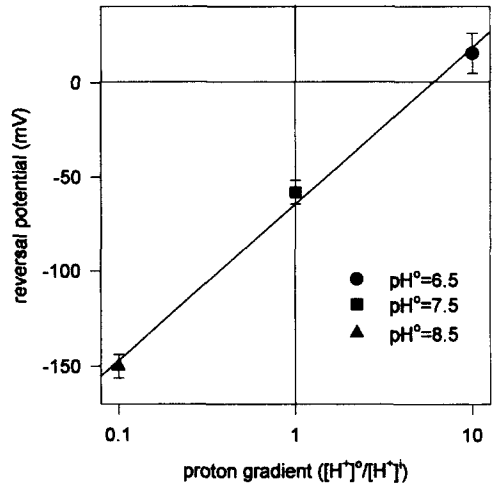

Fig. 2. Dependence of the pump reversal potentials on transmembrane pH gradient. The straight line represents a linear regression fit of a modified Nernst-Equation (Eq. (1)) to the data points. The fitted parameters are: $U_o = -64.2 \pm 4.4$ mV, $z^* = 0.71 \pm 0.05$.

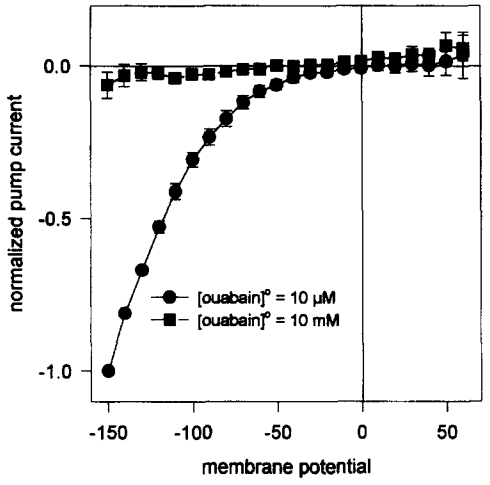

Fig. 3. Dependence of the proton pump currents on intracellular ATP. Voltage dependencies of the normalized differences between currents in the presence and absence of 5 mM intracellular ATP (normalized to the inward current in presence of 10 µM extracellular ouabain at −150 mV, mean current −30.5 ± 5.8 pA, inside-out configuration, mean ± S.E.M., $n = 9$, *Torpedo* pump).

inside-out giant patches were performed. The experimental procedure was to examine ATP-activated currents in the presence of 10 µM or 10 mM extracellular ouabain with a pipette medium (extracellular medium) that is appropriate to activate the proton currents (pH 6.5, 0 Na^+, 0 K^+). Fig. 3 demonstrates that in presence of extracellular ouabain there are nearly no ATP-dependent currents detectable. With 10 µM extracellular ouabain there is a large ATP-activated current, similar in shape and magnitude to the inward rectifying proton current recorded in the outside-out configuration. This result excludes the possibility of a backward running pump mode that transports protons inward.

3.3. *Influence of intracellular Na^+ and K^+ ions on the proton current*

In order to examine the influence of intracellular Na^+ and K^+ ions on the proton pump current, the voltage dependence of proton currents under different intracellular ion conditions was analyzed in the inside-out configuration. Fig. 4 demonstrates that there is no dramatic change in ATP-activated inward current no matter whether Na^+ or K^+ is the only monovalent intracellular cation species. In the presence of intracellular Na^+ ions but not in the presence of intracellular K^+ ions, a small outward current could be measured at positive membrane potentials. This indicates a possible involvement of outward transport of sodium in this potential range.

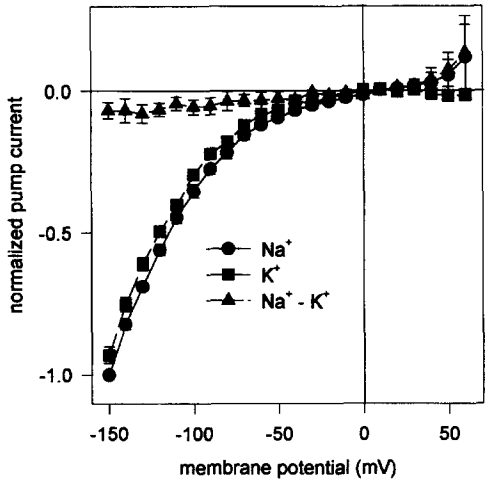

Fig. 4. Influence of intracellular cations on the inward pump current. Voltage dependencies of the normalized ATP-activated currents in the presence of intracellular Na^+ or K^+ ions (circles and squares) and the difference of both (triangles). The currents were normalized to the ATP-activated inward current in the presence of intracellular Na^+ ions at −150 mV (mean current −34.7 ± 10.8 pA, inside-out configuration, mean ± S.E.M., $n = 3$, *Torpedo* pump).

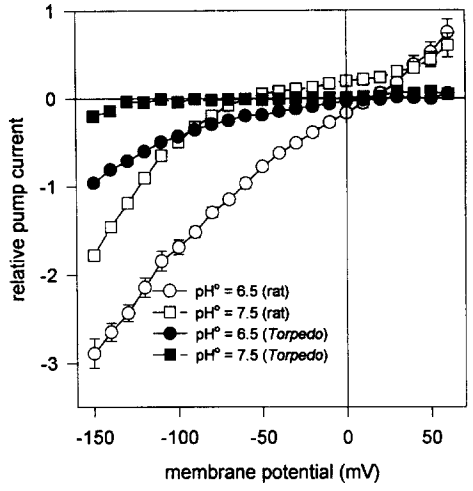

Fig. 5. Comparison between the inward currents in Na^+- and K^+-free extracellular solution generated by the rat or *Torpedo* pump. The *I–V*-dependencies were normalized to the K^+-activated (5 mM) outward current of the respective experiment at −50 mV.

3.4. Comparison between proton currents of Torpedo-(α_1 / β_1) and rat-α_1 / mouse-β_1 pump

The present work was carried out on two different pump species where only the rat-α/mouse-β pump was used for the evaluation of the reversal potentials (see Section 1). With respect to intracellular ATP activation and the influence of intracellular ion composition, both pump species show qualitatively the same characteristics. Only the amount of current for a given extracellular pH differed. In Fig. 5 the comparison between the two species at pH 6.5 and 7.5 is shown. *I–V*-dependencies at pH 8.5 are not included because of the fact that the *Torpedo* pump generates nearly no current at pH values higher than 7.8.

4. Discussion

In a previous publication dealing with pump currents in Na^+- and K^+-free extracellular media, we speculated about the origin of the inward current [4]. At that time it was not clear whether a passive flux of protons or an active transport of protons or $BaCl_2$ is responsible for the observed currents. In a recently published paper, Wang and Horisberger demonstrated that an inward pump current can also occur when the extracellular Ba^{2+} ions are replaced by K^+ channel blockers like quinidine and tolbutamide [5]. Therefore, they concluded that protons and not Ba^{2+} ions are the charge carrying ions for the inward current. The dependence of the pump inward currents on K^+ channel blockers they interpreted with a block of K^+-selective channels: an efflux of K^+ ions through the channels could increase the local potassium concentration close to the membrane and inactivate the proton conductance of the Na^+/K^+-ATPase. The results presented in this work do not support this interpretation. All experiments were performed in K^+-free conditions on both sides of the membrane patch but nevertheless extracellular Ba^{2+} ions were still needed to activate the inward pump current. This discrepancy could possibly be explained by a more direct influence of K^+ channel blockers on the Na^+/K^+-ATPase. This hypothesis is supported by the fact that the interaction of extracellular ions with the protein can be described by the passage of transported ions through a narrow and cation-selective access channel to reach their binding sites [3,11]. Potassium channel blockers could then possibly interact with the porelike access channel that is functionally related to the pore-forming region of K^+-channels.

Wang and Horisberger [5] came to the conclusion that their findings on the dependence of reversal potentials on external pH support the hypothesis of a passive proton conductive pathway formed by the Na^+/K^+ pump. Nevertheless, they could not exclude a transport mechanism like electrogenic Na^+/H^+ exchange. Indeed our results are in favor of a more complex transport cycle than a simple passive mechanism.

First of all the need for intracellular ATP for the activation of the proton current indicates more a reaction cycle rather than a Na^+/K^+-ATPase arrested in E2 conformation forming a proton pore as suggested from Wang and Horisberger. In addition the existence of a negative reversal potential ($U_0 = -58 \pm 5.4$ mV) in the absence of a transmembrane proton gradient is not expected for a passive proton conductance (reversal at 0 mV without a gradient). The result that the shift of

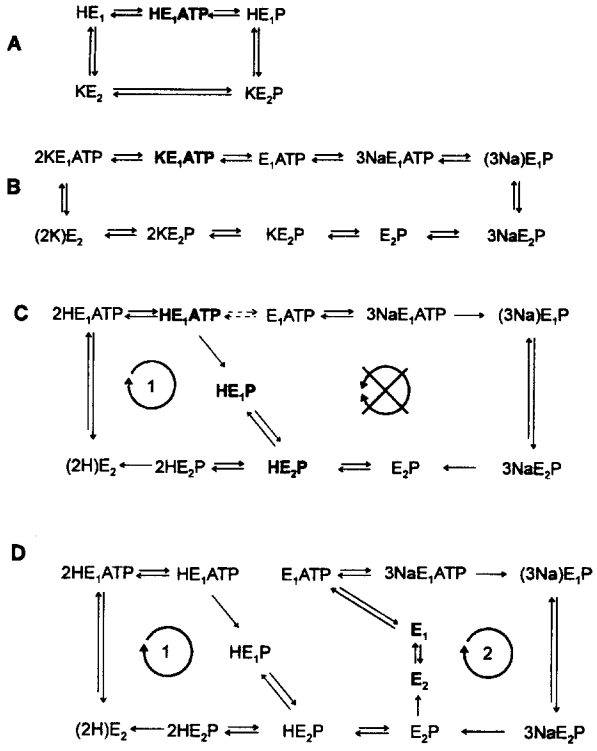

Fig. 6. Reaction schemes of Na^+/K^+-ATPase, H^+/K^+-ATPase and a combination of both. A: Reaction scheme of the gastric H^+/K^+-ATPase [21] B: Albers-Post reaction scheme of the Na^+/K^+-ATPase [22,23]. C: Combination of scheme A and B with extracellular protons replacing extracellular K^+ ions. The conformation HE_1P between HE_1ATP and HE_2P (bold typed) is derived from the reaction scheme of the H^+/K^+-ATPase. D: Final reaction scheme to describe the Na^+/K^+-ATPase generated pump currents in absence of extracellular Na^+ and K^+ ions. The conformations E_1 and E_2 connecting E_1ATP with E_2P are already known for the uncoupled Na^+ efflux mode of the Na^+/K^+-ATPase [15,16].

the reversal potential by 82.7 ± 4.4 mV per 10-fold increase of extracellular proton concentration is described by an effective valency of $z^* = 0.71 \pm 0.05$ also does not point to a passive mechanism.

On the other hand even an unbranched reaction cycle involving protons in transport would give a valency of $z^* = 1$ for the shift in reversal potential. Therefore, we developed a branched reaction cycle that is able to describe our data.

4.1. Hypothetical reaction cycle

The Na^+/K^+-ATPase and the gastric H^+/K^+-ATPase have an amino acid sequence homology of more than 60% [12,13]. Both pumps are functional as a heterodimer composed of an α- and a β-subunit. It has been shown that the β-subunit of H^+/K^+-ATPase can bind to the α-subunit of the Na^+/K^+-ATPase and form a functional transporter [14]. Also the kinetic description of both transporters is in principle similar. Fig. 6A and Fig. 6B show the reaction schemes of both ATPases.

Comparison of the two reaction schemes shows that the HE_1ATP conformation is common to both schemes if the sodium pump binds extracellular protons instead of K^+ ions. Postulating that the Na^+/K^+-ATPase follows the H^+/K^+ reaction cycle after reaching this conformation one comes to a scheme that combines those of Na^+/K^+-ATPase and of H^+/K^+-ATPase (Fig. 6C). If one now introduces the reaction steps for the uncoupled Na^+ efflux mode of the Na^+/K^+-ATPase [15,16], one ends up with the reaction scheme depicted in Fig. 6D. This scheme incorporates 2 unidirectional cycles that generate either inward (cycle 1) or outward current (cycle 2). Under the conditions the presented experiments were performed (absence of intracellular ADP and P_i, absence of extracellular K^+ and Na^+ ions), none of both loops can reverse. Then the pump reversal is not generated by the reversal of a single loop but by a voltage-dependent distribution between the two unidirectional forward or backward running cycles. In case of presence of intracellular Na^+ ions voltage-dependent extracellular proton binding determines the probability of going through cycle 1 (influx of protons, inward current) or 2 (outward transport of Na^+ ions, outward current). This description of a voltage-dependent binding or a voltage-dependent concentration of protons at the binding site is analogous to the voltage-dependent activation by extracellular K^+ ions [3,11].

Table 1
Fitted parameters for the nonlinear least square fits shown in Fig. 1

	I_i^{max}	I_o^{max}	K_l^o [M]	K_f^o [M]	z_l^*	z_f^*
Fig. 1, Eq. (2)	−2.81	0.35	$5.6 * 10^{-9}$	$4.3 * 10^{-9}$	0.92	0.56

The meaning of the fitted parameters are: I_i^{max}: maximal normalized inward current. I_o^{max}: maximal normalized outward current. K_l^o and K_f^o: Apparent half-maximal activating extracellular proton concentrations at 0 mV (see Appendix). z_l^* and z_f^*: effective valencies for extracellular proton binding.

A mathematical description of this kinetic model leads to an expression for a voltage-dependent pump current that is modulated by extracellular pH (see appendix):

$$I_p = \frac{I_o^{max}}{1 + \frac{[H^+]^2}{K_l^0 K_f^0} e^{-(z_l^* + z_f^*)FU/RT}} + \frac{I_i^{max}}{1 + \frac{K_l^0}{[H^+]} e^{z_l^* FU/RT} + \frac{K_l^0 K_f^0}{[H^+]^2} e^{(z_l^* + z_f^*)FU/RT}} \quad (2)$$

To test whether the derived current equation is able to describe the experimental data, a simultaneous nonlinear least squares fit of Eq. (2) to the data presented in Fig. 1 was performed. Fig. 1 shows the I–V-dependencies of pump currents at the different extracellular pH-values and the overlaid fitted curves. Although 6 parameters are varied during the curve-fitting process, the fitted parameters are in a wide range independent of the initial values and the parameter set in Table 1 is the only one that could be found to describe the experimental data. Another argument for the relevance of the parameters is that the calculated values for the effective valencies are in line with earlier findings [13].

In Eq. (2), z_l^* and z_f^* are no more valencies of the transported ion species but effective valencies of proton binding and can, therefore, have a value different from 1. The absolute values for the reversal potentials depend not explicitly on the transmembrane proton concentration but are determined by the maximal outward and inward currents I_o^{max} and I_i^{max}, the apparent half maximal activating proton concentrations at 0 mV K_l^0 and K_f^0 and the effective valencies z_l^* and z_f^*. This means that even in the absence of a transmembrane proton gradient there is the possibility to get a non-zero reversal potential.

The intracellular Na^+-concentration was kept constant during the pH-dependent reversal potential measurements (Fig. 1). Reducing the intracellular Na^+-concentration to non-saturating values would cause a reduced maximal outward current through cycle 2 and therefore a shift of the reversal potentials to more negative values. Experiments that would give information about the dependence of the pump reversals on intracellular ion compositions are therefore planned.

In summary, the presented study gives a possible kinetic explanation for the observed characteristics of the Na^+/K^+-ATPase under K^+- and Na^+-free extracellular conditions. The role of the extracellular Ba^{2+} ions or K^+ channel blockers as a regulator of the pump currents under these conditions nevertheless remains unclear and needs further investigation. A denaturation of the pump protein caused by the extracellular Ba^{2+} ions can be excluded from the findings of earlier measurements, demonstrating that the function of the sodium pump is not affected by extracellular Ba^{2+} ions [17].

Acknowledgements

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Appendix A

To derive the flux equations for the proton and voltage-dependent pump currents in Na^+- and K^+-free extracellular medium, a simplification of the reaction scheme shown in Fig. 6D was performed. If one replaces the conformations ranging from $(2H)E_2$ to HE_1P by E_i and the conformations ranging from E_2 to $(3Na)E_1P$ by E_o (Fig. 7A), one gets a simplified reaction diagram (Fig. 7B) that is equivalent to the complete reaction scheme. The rate constants k_2, k_3, k_A, k_B and k_C incorporate the voltage- and proton concentration-independent rate constants connecting the conformational states that were substituted by E_i and E_o. For further simplification, the bidirectional reaction between HE_2P and $2HE_2P$ was replaced by the unidirectional rate constant k_1 [18]

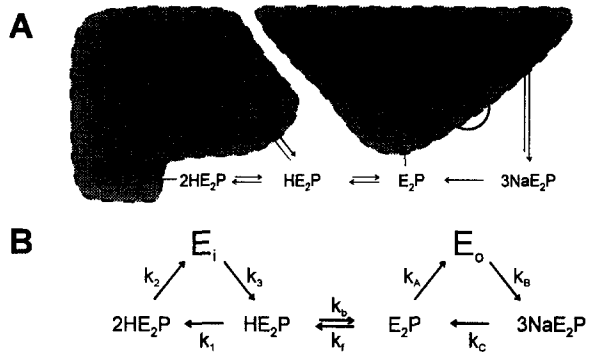

Fig. 7. A: Reaction scheme for the observed pump currents in Na^+- and K^+-free extracellular solutions. The conformational states in the shaded areas of cycle 1 and 2 are combined to the conformational states E_i and E_o in B. B: Simplified reaction scheme regarding explicitly the interaction of the protein with extracellular cations.

Using the formalism of King and Altmann [19], one gets expressions for the velocity of cycle 1 (ν_i) and cycle 2 (ν_o):

$$\nu_i = k_1 \cdot HE_2P = \frac{k_1 k_2 k_3 k_B k_C k_f}{k_B k_C k_f (k_2 k_3 + k_1 k_3 + k_1 k_2) + k_2 k_3 k_b (k_B k_C + k_A k_C + k_A k_B)} \tag{A.1}$$

and

$$\nu_o = k_A \cdot E_2P = \frac{k_2 k_3 k_A k_B k_C k_b}{k_B k_C k_f (k_2 k_3 + k_1 k_3 + k_1 k_2) + k_2 k_3 k_b (k_B k_C + k_A k_C + k_A k_B} \tag{A.2}$$

The rate constants k_1 and k_f depend on the voltage-dependent proton concentrations at the binding sites [20]:

$$k_1 = k_1^0 \cdot [H^+] \cdot e^{-z_1^* FU/RT} \tag{A.3}$$

$$k_f = k_f^0 \cdot [H^+] \cdot e^{-z_f^* FU/RT} \tag{A.4}$$

where k_1^0 and k_f^0 are the proton binding rate coefficients at 0 mV, $[H^+]^o$ is the extracellular proton concentration and z_1^*, z_f^* are the effective valencies of extracellular proton binding. F, U, R and T have their usual meanings.

For infinite negative potentials ν_i becomes maximal:

$$\nu_i^{max} = \frac{k_2 k_3}{k_2 + k_3} \tag{A.5}$$

For infinite positive potentials becomes maximal:

$$\nu_o^{max} = \frac{k_A k_B k_C}{k_A k_B + k_B k_C + k_A k_C} \tag{A.6}$$

Rearranging Eq. A.1 gives:

$$\nu_i = \frac{k_2 k_3}{\frac{k_2 k_3}{k_1} + k_3 + k_2 + \frac{k_2 k_3 k_b (k_B k_C + k_A k_C + k_A k_B)}{k_1 k_B k_C k_f}} \tag{A.7}$$

and with Eq. A.5:

$$\nu_i = \frac{\nu_i^{max}}{1 + \frac{1}{k_1}\left(\frac{k_2 k_3}{k_2 + k_3}\right) + \frac{1}{k_1}\left(\frac{k_2 k_3}{k_2 + k_3}\right) \cdot \frac{1}{k_f}\left(\frac{k_b (k_B k_C + k_A k_C + k_A k_B)}{k_B k_C}\right)} \tag{A.8}$$

and:

$$\nu_i = \frac{\nu_i^{max}}{1 + K_1 + K_1 K_f} \tag{A.9}$$

with:

$$v_i^{max} = \frac{k_2 k_3}{k_2 + k_3} \quad K_1 = \frac{(k_2 k_3)}{k_1 (k_2 + k_3)}$$

$$K_f = \frac{k_b}{k_f} \left(\frac{(k_B k_C + k_A k_C + k_A k_B)}{k_B k_C} \right)$$

The velocity is proportional to the generated current. Together with equation A.3 and A.4 one can write:

$$I_i = \frac{I_i^{max}}{1 + \frac{K_1^0}{[H^+]} \cdot e^{z_1^* FU/RT} + \frac{K_1^0 K_f^0}{[H^+]^2} \cdot e^{(z_1^* + z_f^*)FU/RT}} \tag{A.10}$$

where K_1^o and K_f^o are the apparent half-maximal activating extracellular proton concentrations at 0 mV.

In an analogous derivation one gets an expression for the voltage and proton concentration-dependent outward current I_o:

$$I_o = \frac{I_o^{max}}{1 + \frac{[H^+]^2}{K_1^0 K_f^0} \cdot e^{-(z_1^* + z_f^*)FU/RT}} \tag{A.11}$$

The total pump current is then:

$$I_p = I_o + I_i = \frac{I_o^{max}}{1 + \frac{[H^+]^2}{K_1^0 K_f^0} \cdot e^{-(z_1^* + z_f^* FU/RT)}} + \frac{I_i^{max}}{1 + \frac{K_1^0}{[H^+]} \cdot e^{z_1^* FU/RT} + \frac{K_1^0 K_f^0}{[H^+]^2} \cdot e^{(z_1^* + z_f^*)FU/RT}} \tag{A.12}$$

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